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TECHNICAL REPORT 1913
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ADMINISTRATIVE INFORMATION

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EXECUTIVE SUMMARY

The U.S. Office of Naval Research (ONR) and Chief of Naval Operations (CNO) (N45) have sponsored research programs to investigate the auditory effects of high-intensity sounds on marine mammals. In addition to auditory effects, these studies reported behavioral reactions as the subjects were exposed to sounds of increasing intensity. The most common reactions were attempts by the subjects to avoid the site of previous noise exposures, or attempts to avoid an exposure in-progress.

Schlundt *et al.* (2000) gave a brief summary of the more significant behavioral changes they observed in dolphins and white whales exposed to intense pure tones. This report presents a more detailed summary of behavioral responses of dolphins and white whales exposed to 1-s tones.

Test sessions were grouped by species and exposure frequency. Within each group, the percentage of sessions in which subjects showed altered behavior was calculated as a function of exposure sound pressure level (SPL) and energy flux density level (EL). Altered behavior was defined as a change from a subject's "normal" behavior observed during baseline sessions without intense sound exposure. The percentage of sessions with altered behavior generally increased with increasing exposure levels. For pooled data at 3, 10, and 20 kHz, exposure ELs corresponding to sessions with 25, 50, and 75% altered behavior were 180, 190, and 199 dB re 1 μPa^2-s, respectively.

Behavioral effects were quantitatively assessed by comparing the time for the subjects to swim from one apparatus (the "S1 station") to another apparatus (the "S2 station"). Unlike behavioral reactions, which could only be assessed subjectively, S1–S2 travel times could be objectively measured. Unfortunately, there was no clear relationship between S1–S2 travel times and exposure SPL.

CONTENTS

1. INTRODUCTION

In response to concerns over the potential effects of underwater noise on marine mammals, the U.S. Office of Naval Research (ONR) and Chief of Naval Operations (CNO) (N45) have sponsored a number of research programs designed to investigate the auditory effects of high-intensity sounds on marine mammals (e.g., Kastak *et al.*, 1999; Schlundt *et al.*, 2000; Finneran *et al.*, 2000a; Finneran *et al.*, 2002; Nachtigall *et al.*, 2003).

The three groups actively researching marine mammal temporary threshold shift (TTS) are located at the Space and Naval Warfare Systems Center, San Diego (SSC San Diego), the University of California Santa Cruz, and the Hawaii Institute of Marine Biology. The groups' hearing test method, species studied, and exposure conditions differ, but the basic procedures are similar: hearing thresholds are measured in trained marine mammals before and after exposure to sounds with various sound pressure levels (SPLs), waveforms, frequencies, and durations to determine the amount of TTS produced. TTS is a temporary hearing loss that completely recovers after some period of time. The amount of TTS (in decibels) is calculated by subtracting the post-exposure hearing threshold from the pre-exposure threshold. A TTS indicates an increase in hearing threshold, which means a decrease in sensitivity (i.e., hearing loss). TTS data from these studies are used to estimate acoustic zones of impact for Navy activities (e.g., DoN, 2001).

The groups conducting TTS research have also noted certain behavioral alterations, or changes from the subjects' trained behaviors, that tend to occur as the subjects are exposed to sounds of increasing intensity. Behavioral alterations often consisted of attempts by the subjects to avoid the site of previous noise exposures (e.g., Schlundt *et al.*, 2000), or attempts to avoid an exposure in-progress (e.g., Kastak *et al.*, 1999). On some occasions, the subjects became aggressive or refused to further participate in the test (Schlundt *et al.*, 2000).

Schlundt *et al.* (2000) presented some of the more significant behavioral changes and exposure levels above which behavioral changes were observed, but they did not provide a detailed analysis or breakdown by exposure SPL. The objective of this study was to present a more detailed summary of the behavioral observations recorded during TTS tests conducted at SSC San Diego with 1-s tones. These experiments were originally reported in Schlundt *et al.* (2000) and Finneran *et al.* (2001, 2003). This report presents the methods used to analyze the behavioral data and the results.

2. BACKGROUND

The SSC San Diego TTS test methodology is described in detail in Schlundt *et al.* (2000) and Finneran *et al.* (2000a, 2002, 2003); however, because the interpretation of the behavioral data depends on the specific test procedures, a description of the TTS methodology is provided.

TTS testing requires three steps: (1) measurement of the subject's pre-exposure hearing threshold, (2) exposure of the subject to an intense underwater sound, called the "fatiguing stimulus," and (3) measurement of the subject's post-exposure hearing threshold. At SSC San Diego, hearing thresholds are measured by training subjects to perform specific actions when they hear certain sounds. Pure tones, or hearing test tones, are played to a subject at various levels and the subject's responses recorded. The hearing threshold is defined as the SPL at which the subject responds 50% of the time.

Figure 1 shows the TTS test setup. The test apparatus contains two underwater test platforms, called "stations." The two stations are referred to as the "S1 station" and the "S2 station." The intense sound exposure occurs at the S1 station. The hearing tests are conducted at the S2 station. Two stations are used to physically separate the hearing test location from the intense sound exposure location. Each station contains underwater sound projectors, hydrophones, a video camera, and a plastic "biteplate" on which the subject is trained to position itself. The biteplate ensures that the subject's head is in a known position with respect to the sound sources.

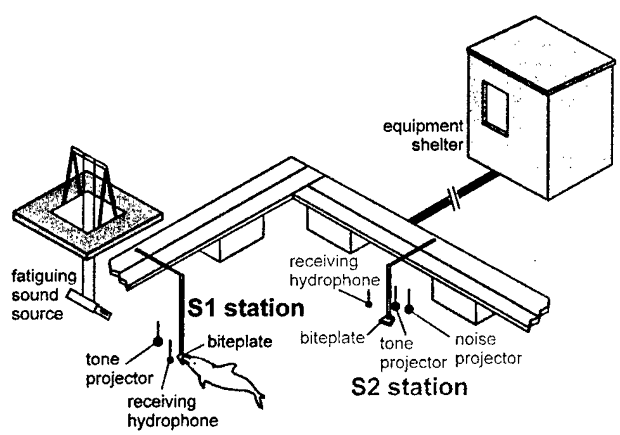

Figure 1. TTS test setup at SSC San Diego.

The test sequence begins with the trainer cueing the subject to dive underwater and position itself on the S1 biteplate. The subject remains at the S1 station until presented with a specific sound referred to as the "S1 release signal" or "S1 signal." When it hears the S1 signal, the subject swims to the S2 station and positions itself on the S2 biteplate. Once at the S2 station, the subject is presented with a number of hearing test tones. The subject is trained to produce an audible response if it detects a hearing test tone and to remain quiet otherwise. After a variable number of tones, the subject is recalled to the surface and given a fish reward.

The sequence described above is repeated until the pre-exposure hearing threshold is obtained. The subject is then cued to the S1 station and exposed to the intense sound. Following the intense sound

exposure, the post-exposure hearing threshold is measured (in a manner similar to the pre-exposure threshold). Pre- and post-exposure thresholds are compared to determine the amount of TTS.

2.1 SCHLUNDT *ET AL.* (2000)

Schlundt *et al.* (2000) reported eight individual TTS experiments. Table 1 lists the fatiguing sound frequencies, exposure levels, and species tested during each experiment (exp. 1 through 8 in Table 1). Fatiguing stimuli durations were 1 s. The experiments were conducted in San Diego Bay. Because of the variable ambient noise in the bay, low-level broadband masking noise was used to keep hearing thresholds consistent despite fluctuations in the ambient noise. The S1 release signal was a 1-s, 141 dB re 1 µPa tone at the same frequency as the fatiguing stimulus. For the actual fatiguing stimulus, the S1 signal level was increased to the desired fatiguing sound level. Fatiguing sound levels generally increased from day to day during each experiment until a measurable TTS (i.e., greater than 6 dB) was observed. Experiments 1 through 8 differed in the exact test sequence. For example, some tests featured "recovery" thresholds measured tens of minutes or hours after the post-exposure threshold [see Schlundt *et al.* (2000) for more details].

Schlundt *et al.* (2000) reported that "behavioral alterations," or deviations from subjects' trained behaviors, occurred as the subjects were exposed to increasing fatiguing stimulus levels. Schlundt *et al.* also reported measurements of the amount of time taken for the subjects to travel from the S1 station to the S2 station after the fatiguing sound exposure. These data [Figure 8 in Schlundt *et al.* (2000)] suggested a relationship between the S1–S2 travel time, behavioral alterations, and the fatiguing stimulus level.

2.2 FINNERAN *ET AL.* (2001, 2003)

Finneran *et al.* (2001, 2003) conducted TTS experiments at SSC San Diego using 1-s duration tones at 3 kHz. The test method was similar to that of Schlundt *et al.* except the tests were conducted in a pool with a very low ambient noise level (below 50 dB re 1 $\mu Pa^2/Hz$), and no masking noise was used. The S1 signal was a sinusoidal amplitude modulated tone with a carrier frequency of 12 kHz, modulating frequency of 7 Hz, and SPL of approximately 100 dB re 1 µPa. An S1 signal distinct from the fatiguing sound allowed true control sessions. Two separate experiments were conducted. In the first (Table 1, exp. 9), fatiguing sound levels were increased from 160 to 201 dB SPL. In the second experiment (Table 1, exp. 10), fatiguing sound levels between 180 and 200 dB re 1 µPa were randomly presented.

Table 1. Fatiguing sound frequencies, exposure levels, and number of subjects. The same two white whales were used for all tests at 3 and 20 kHz. Four different dolphins were used for the 3- and 20-kHz tests (some dolphins participated in more than one experiment).

Exp.	Frequency (kHz)	Levels (dB re 1 μPa)	Subjects (dolphin/w. whale)
1	20	160–197	2/0
2	75	160–194	2/0
3	3	160–202	2/2
4	10	180–197	2/2
5	20	180–201	2/2
6	20	178–202	2/2
7	3	180–201	2/2
8	0.4	179–193	2/2
9	3	160–201	2/0
10	3	180–200	2/0

3. METHODS

3.1 BEHAVIORAL ALTERATIONS

Behavioral observations recorded by the trainers or test coordinators during the Schlundt *et al.* (2000) and Finneran *et al.* (2001, 2003) experiments were examined. A total of 193 exposure sessions (fatiguing stimulus level > 141 dB re 1 μPa) were evaluated from Schlundt *et al.* (2000) and 21 exposure sessions from Finneran *et al.* (2001, 2003). Each exposure was put into one of the following nine exposure groups: 160±3, 170±3, 175±2, 180±2, 186±3, 192±2, 196±1, 199±1, and 201±1 dB re 1 μPa. The exposure groups and ± ranges were based on the distribution of the actual exposure SPLs. Exposures falling within the bounds outlined above were considered to have been at the center of the SPL range. For example, an exposure at 188 dB re 1 μPa would be put into the 186±3 group and from this point on considered to be at 186 dB re 1 μPa. All exposures had durations of 1 s.

The observations were used to subjectively assess a subject's behavior during the session. This assessment relied upon detailed knowledge of the subject's "normal" behaviors observed during baseline sessions conducted with no intense sound exposures. The main types of "altered behaviors" observed during the tests were:

- Committing more false alarms than normal during a hearing test
- Leaving the S2 station before signaled
- Not swimming to the S2 station after receiving the S1 release signal
- Returning to the S1 station after an S1 signal or fatiguing sound exposure (required an additional S1 release signal to go to S2)
- Tail slapping, "jaw popping" (see Finneran *et al.*, 2000b)
- Departing the S1 station in a direction away from the S2 station
- "Floating" to the S2 station, sluggish behavior
- Swimming erratically around test enclosure
- Ignoring the trainer, floating in test enclosure corner
- Vocalizing after the fatiguing stimulus exposure
- Positioning improperly on the S1 biteplate
- Requiring additional cues from trainer before going to S1 station
- Leaving the S1 station before the S1 signal
- Refusing to return to the S1 station
- Attacking the S1 station

The behavioral alterations are roughly arranged in order of least severe to most severe. The subjective assessment was used to categorize the subject's behavior in each session as "normal" or "altered." Altered behaviors were not restricted to the time period after the fatiguing sound exposure—in some cases behavioral changes (e.g., leaving the S1 station early) occurred before the fatiguing sound exposure. The subjective analysis in this report was, in general, more liberal than that performed by Schlundt *et al.* (2000), who reported mostly significant behavioral changes directly resulting from exposures; there may therefore be some differences between the results. The results presented here also include additional data not presented by Schlundt *et al.* (2000).

After categorizing each session as altered or normal behavior, sessions were grouped according to species and exposure frequency. For each species and frequency combination, the percentage of sessions with altered behavior was calculated for each exposure SPL group. The relatively small number of exposures for each subject prevented analyzing the data on a per subject basis. Data are

reported for dolphins and white whales and for both species pooled. A probit analysis technique (Finney, 1971) was used to fit smooth dosage-response curves to the percent altered behavior versus SPL data for the pooled dolphin/white whale data sets, except for the 0.4-kHz data, which could not be properly fit (see section 5.1).

3.2 S1–S2 TRAVEL TIMES

Exposures were categorized as outlined above and sessions were grouped according to species and fatiguing stimulus frequency. For each species group/exposure SPL combination, the mean S1–S2 travel time was calculated. Mean travel times from baseline test sessions reported by Schlundt *et al.* (2000) were also analyzed. The baseline travel times were from a random sample of 15 S1–S2 intervals over a minimum of 3 test days (Schlundt *et al.*, 2000).

4. RESULTS

4.1 BEHAVIORAL ALTERATIONS

Figures 2 through 8 show the results of the subjective behavior analysis. Each plot shows the percentage of sessions with altered behavior at each exposure SPL. Figures 2 through 6 show the data for 0.4, 3, 10, 20, and 75 kHz individually. Figures 7 and 8 pool the data at 3, 10, and 20 kHz and 0.4, 3, 10, 20, and 75 kHz, respectively. Each figure has three panels. The top panel shows the pooled results for both dolphins and white whales; the middle and bottom panels show the white whale and dolphin data separately. The exception to this is Figure 6, which has only two panels since white whales were not tested at 75 kHz. The solid lines show the curve-fits resulting from the probit analysis. The 0.4-kHz data suggested a decreasing percent altered behavior with increasing exposure SPL and thus could not be fit with the classic dosage response curves as seen in Figures 3 through 8.

The numbers above the bars in the lower panels indicate the number of (total) exposure sessions for each species/frequency group. The pooled data show the percentage of sessions with altered behavior after the data were pooled, not the average of the original (unpooled) data. For example, at 0.4 kHz, 180 dB re 1 μPa, altered behaviors were noted in 2 of 4 (50%) white whale sessions and 2 of 3 (66.6%) dolphin sessions. When the dolphin and white whale data were pooled, the percentage became 4 of 7 or 57%. The same method was used to pool data from multiple frequencies: percentages were re-calculated by dividing the total number of sessions with altered behavior by the total number of exposure sessions.

Exposure SPLs corresponding to specific percentages of sessions with altered behavior may be found by interpolating within Figures 3 through 8. Example results are displayed in Table 2 for percentages of 25, 50, and 75%.

Table 2. Exposure SPLs (dB re 1 μPa) corresponding to 25, 50, and 75% of sessions with altered behavior for the different frequency groups. Results are for the pooled white whale and dolphin data.

Frequency group (kHz)	25%	50%	75%
3	184	192	200
10	177	182	186
20	183	191	200
75	175	181	188
3, 10, 20	180	190	199
0.4, 3, 10, 20, 75	173	189	204

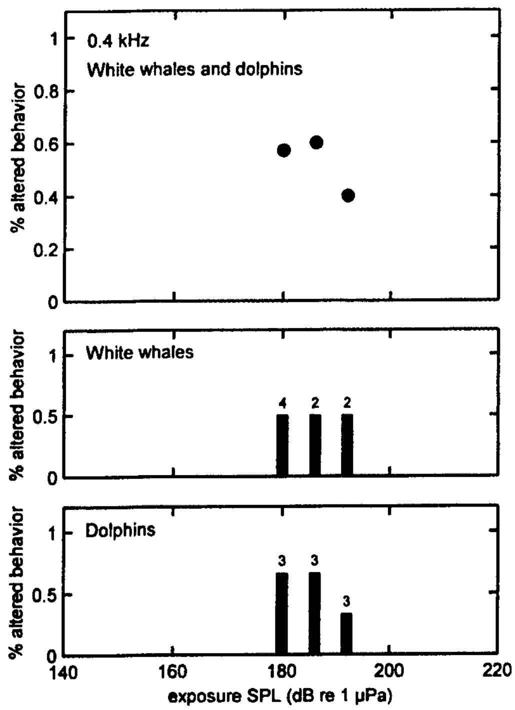

Figure 2. Altered behavior as a function of exposure SPL at 0.4 kHz.

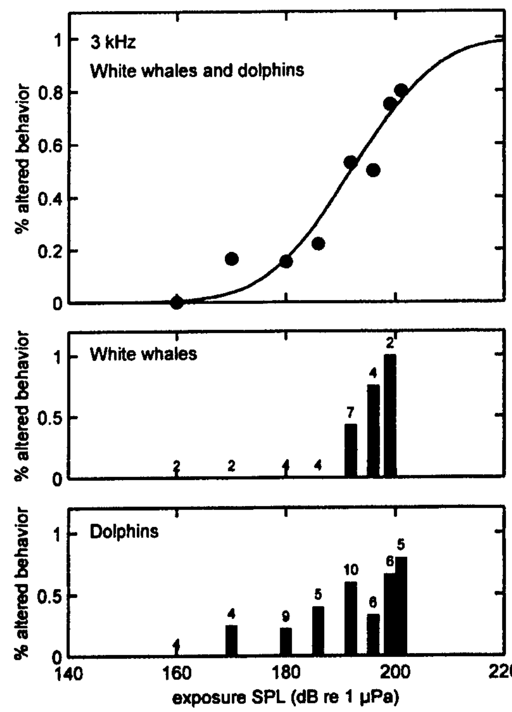

Figure 3. Altered behavior as a function of exposure SPL at 3 kHz.

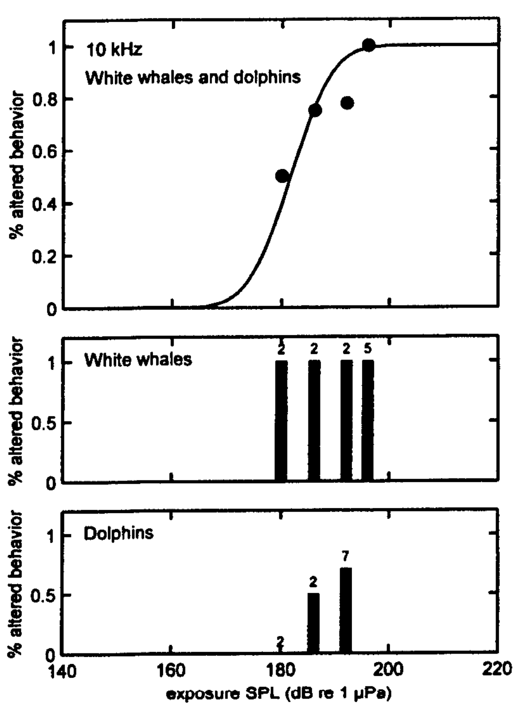

Figure 4. Altered behavior as a function of exposure SPL at 10 kHz.

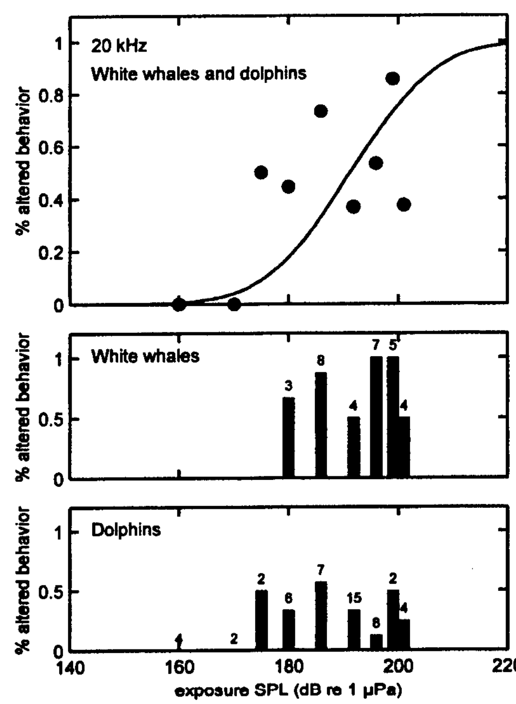

Figure 5. Altered behavior as a function of exposure SPL at 20 kHz.

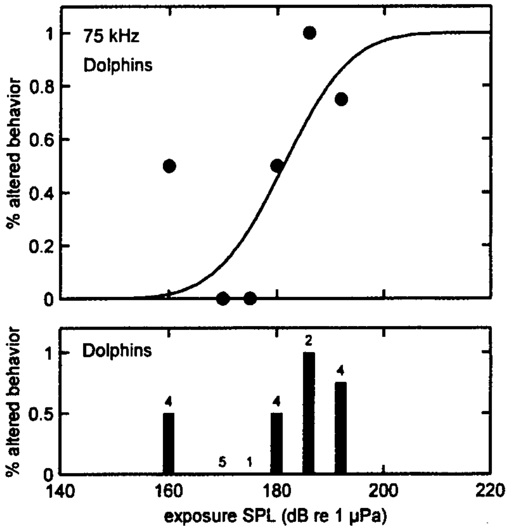

Figure 6. Altered behavior as a function of exposure SPL at 75 kHz.

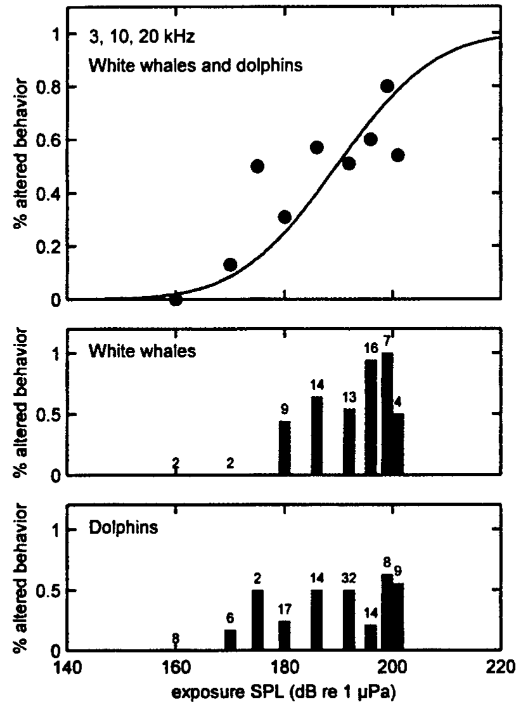

Figure 7. Altered behavior as a function of exposure SPL at 3, 10, and 20 kHz.

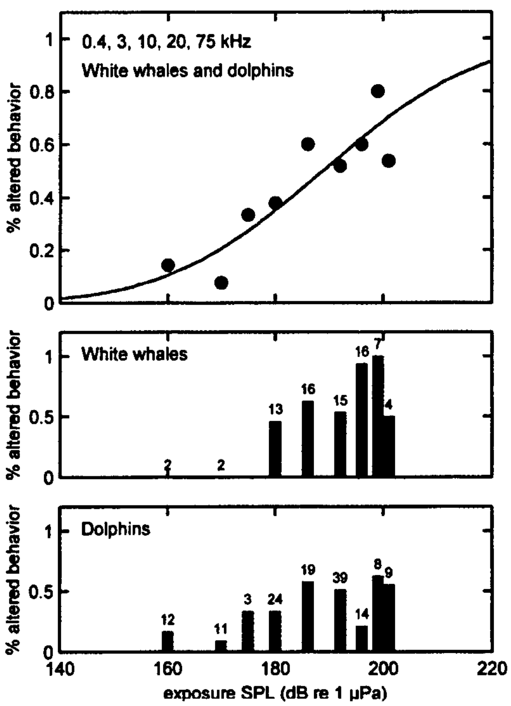

Figure 8. Altered behavior as a function of exposure SPL at 0.4, 3, 10, 20, and 75 kHz.

4.2 S1–S2 TRAVEL TIMES

Figures 9 through 15 show the mean S1–S2 travel times as functions of exposure SPL for different exposure frequencies. Figures 9 through 13 show the data for 0.4, 3, 10, 20, and 75 kHz individually. Figures 14 and 15 pool the data at 3, 10, and 20 kHz and 0.4, 3, 10, 20, and 75 kHz, respectively. Each figure has three panels. The top panel shows the pooled results for both dolphins and white whales; the middle and bottom panels show the white whale and dolphin data separately. The exception to this is Figure 13, which has only two panels since white whales were not tested at 75 kHz. The travel time data were pooled in a manner similar to the subjective behavioral data: means were re-calculated from the pooled data. The error bars indicate standard deviations. The "base" data point represents the mean and standard deviation from a random sample of 15 S1–S2 intervals over a minimum of three baseline test days (Schlundt *et al.*, 2000). Note that the ordinate limits are not uniform in all figures.

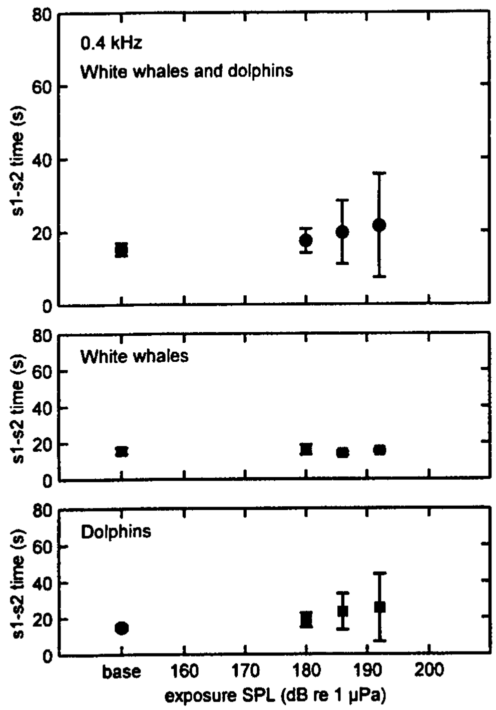

Figure 9. Mean S1–S2 travel times from baseline and exposure sessions at 0.4 kHz. Error bars indicate standard deviations.

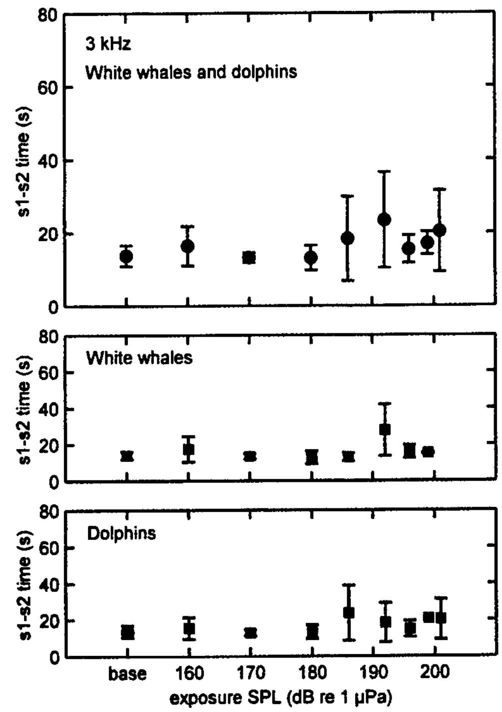

Figure 10. Mean S1–S2 travel times from baseline and exposure sessions at 3 kHz. Error bars indicate standard deviations.

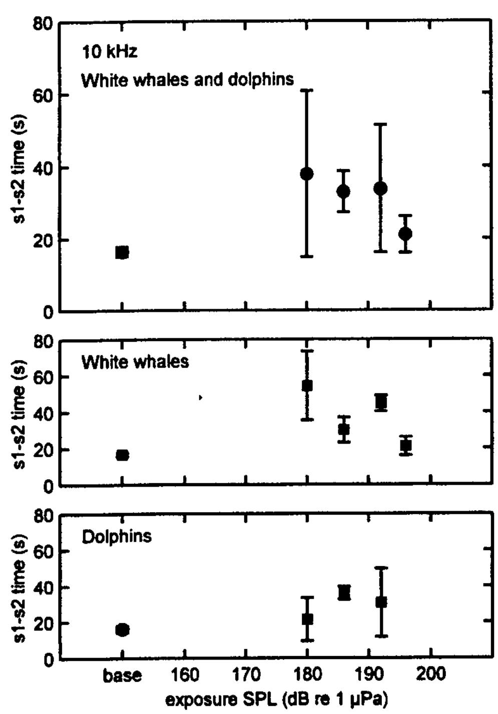

Figure 11. Mean S1–S2 travel times from baseline and exposure sessions at 10 kHz. Error bars indicate standard deviations.

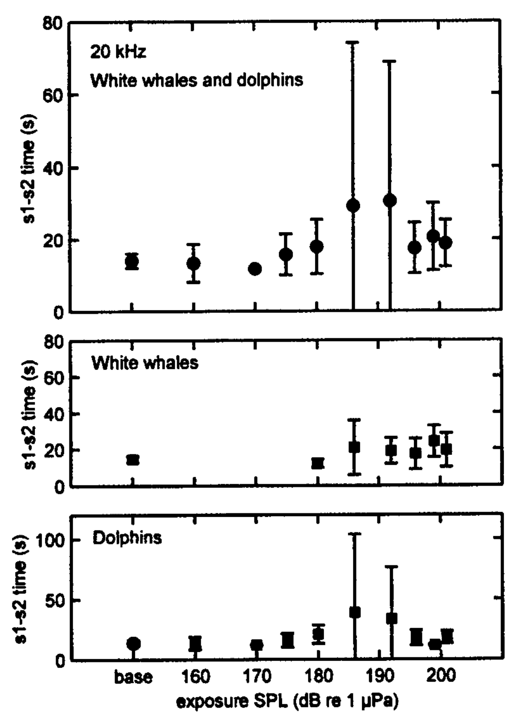

Figure 12. Mean S1–S2 travel times from baseline and exposure sessions at 20 kHz. Error bars indicate standard deviations.

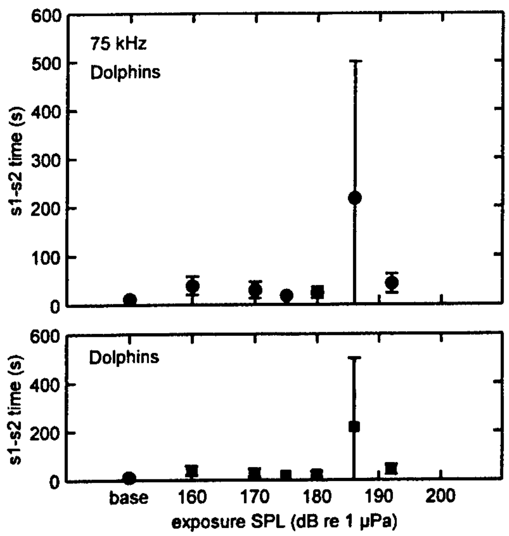

Figure 13. Mean S1–S2 travel times from baseline and exposure sessions at 75 kHz. Error bars indicate standard deviations.

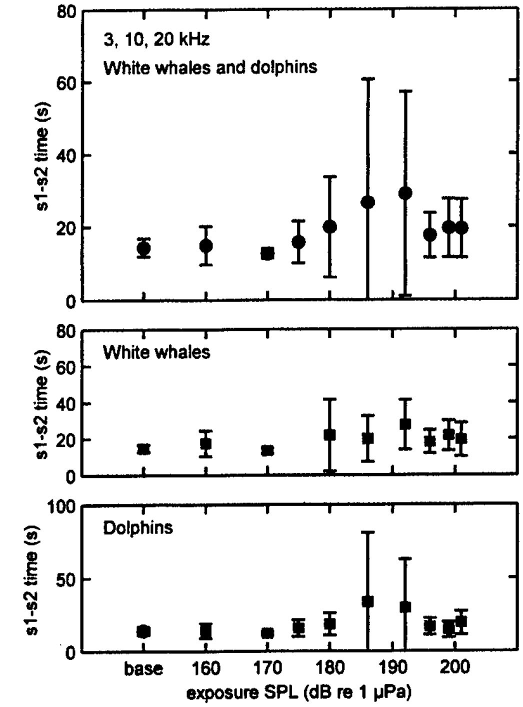

Figure 14. Mean S1–S2 travel times from baseline and exposure sessions at 3, 10, and 20 kHz. Error bars indicate standard deviations.

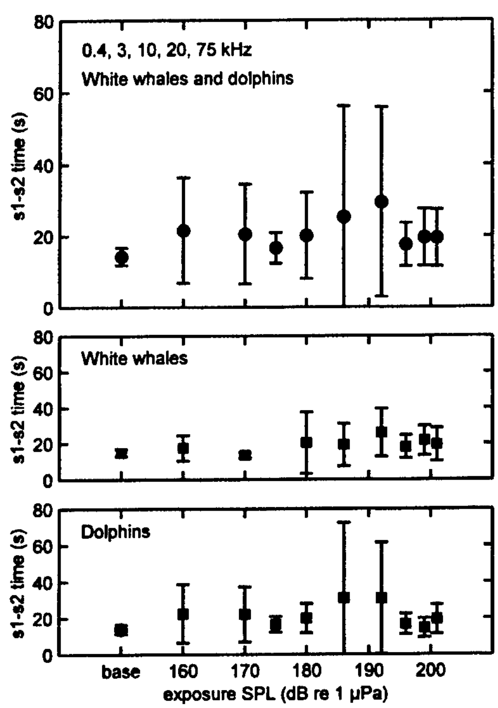

Figure 15. Mean S1–S2 travel times from baseline and exposure sessions at 0.4, 3, 10, 20, and 75 kHz. Error bars indicate standard deviations.

5. DISCUSSION

5.1 BEHAVIORAL ALTERATIONS

The behavior of a subject during intense sound exposure experiments was compared to the subject's normal behaviors to determine whether a subject exhibited altered behavior during a session. In this context, altered behavior means a deviation from a subject's normal trained behaviors. The subjective assessment was only possible because behavioral observations were made with the same subjects during many baseline hearing sessions with no intense sound exposures. This allowed comparisons to be made between how a subject normally acted and how it acted during test sessions with fatiguing sound exposures.

Subjectively categorizing each exposure session as normal or altered behavior allowed the percentage of sessions with altered behavior to be calculated as a function of the exposure SPL. Figures 2 through 8 show the percentage of sessions with altered behavior as a function of the exposure SPL for different species/frequency combinations. As reported by Schlundt *et al.* (2000), instances of altered behavior generally began at lower exposures than those causing TTS; however, there were many instances when subjects exhibited no altered behavior at levels above the onset-TTS levels. The 3-, 10-, and 20-kHz data generally show increasing percentages with increasing exposure SPL. The 0.4-kHz percentages do not increase with frequency; however, this is likely a reflection of the sparse data rather than a true relationship between increasing exposure and behavioral effects. The 75-kHz data tend to increase with exposure SPL but possess a great deal of variability.

Schlundt *et al.* (2000) reported minimum exposure levels required to produce behavioral alterations. The subjective analysis performed for this report was more liberal; changes from the subject's normal behaviors were considered to represent altered behavior regardless of whether that change could be explicitly tied to the fatiguing sound exposure. The more liberal approach was considered appropriate for this report because the data presentation method (percentage of sessions with altered behavior) helps to prevent misinterpreting the data. For example, presenting the minimum level at which any change was observed may be misleading if most occurrences of altered behavior were at much higher levels. Figures 2 through 8 keep the individual data values in perspective with respect to the effects at other exposure SPLs.

5.1.1 Limitations

Interpretation and extrapolation of the data shown in Figures 2 through 8 should be done with caution. These data represent behaviors of particular trained subjects in controlled circumstances. Behavioral reactions do not depend solely on the sound exposure, but also may vary with the subject's prior experience and motivational state. A great deal of variability may exist between subjects. Some subjects were more tolerant of the intense sound exposures than others. Some subjects were only used in one experiment while others were tested multiple times. Since all subjects were not tested at each exposure frequency, this may have skewed the data at some frequencies; one would expect more representative results from frequencies tested with more individuals.

Responses of trained, experienced subjects are not necessarily applicable to wild and/or naïve animals. Experienced and/or food-motivated subjects may tolerate higher sound levels than inexperienced or unmotivated animals. It is also possible that prior experiences may have made some subjects less tolerant of the sound exposures.

Because the TTS experiments were not designed to measure behavioral effects, certain aspects of the experimental design created confounds that affect the extrapolation of these data to other scenarios. For example, exposure levels generally started relatively low and increased over time until

the subject exhibited a measurable TTS. This makes it difficult to attribute a particular behavioral reaction to a specific exposure, rather than to learning and the cumulative effect of increasing exposure levels over a period of days or weeks. Some behavioral reactions (e.g., refusing to station at the S1 biteplate) occurred before the fatiguing sound exposure (also reported in Finneran *et al.*, 2003). This shows that the subjects' prior experiences affected their responses during the tests. This also blurs potential cause-and-effect relationships between the intense sound exposure and the observed behavioral reactions.

During experiments 1 through 8, the fatiguing sound also acted as the S1 release signal (although at a higher SPL than normal). This test approach prevented control sessions where the test procedures are identical to the exposure sessions except there is no fatiguing sound exposure. This is a potential confound if subjects discriminated between the S1 release signal at 141 dB re 1 µPa and more intense fatiguing stimuli at higher levels. A subject may not have recognized the fatiguing sound as the cue to go to S2 (resulting in an increased S1–S2 time) simply because it was at a much higher level than normal, not because the received level was aversive or bothersome.

Another potential problem arises from the lack of data at lower levels in many experiments. The TTS experiments concentrated exposures near SPLs that were capable of inducing a TTS. In several experiments, there were no exposures below 180 dB re 1 µPa. In one case (white whales at 10 kHz), behavioral alterations were observed during both sessions at 180 dB re 1 µPa (the lowest exposure level) and all higher levels. It is unknown if behavioral alterations would have been observed at lower exposure levels if they had been tested.

5.2 S1–S2 TRAVEL TIMES

S1–S2 travel time measurements enable quantitative comparisons between baseline and exposure sessions at various SPLs. There was a large variation in the S1–S2 travel times for some exposure conditions. Some behavioral reactions, such as ignoring the trainer or requiring additional S1 release signals before going to S2, dramatically increased the measured travel time.

Schlundt *et al.* (2000) showed significant differences between mean S1–S2 travel times for baseline and exposure session *with altered behavior*. In this report, all exposure sessions are included, rather than just exposure sessions with altered behavior. Figures 9 through 15 do not indicate any obvious relations between exposure SPL and mean S1–S2 travel times. The difference between the Schlundt *et al.* (2000) results (significant differences between baseline and exposure sessions with altered behavior) and the current result (no difference between baseline and exposure sessions) arises because the S1–S2 travel time was one of the factors used to categorize a session as altered or normal behavior. Although the sessions judged by Schlundt *et al.* to exhibit altered behavior had larger mean S1–S2 times than the baseline sessions, the S1–S2 travel time cannot be used as a predictor of altered behavior.

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14. ABSTRACT

This report presents behavioral responses of dolphins and white whales exposed to 1-s tones.

The U.S. Office of Naval Research (ONR) and Chief of Naval Operations (CNO) (N45) have sponsored research programs to investigate the auditory effects of high intensity sounds on marine mammals. In addition to auditory effects, these studies reported behavioral reactions as the subjects were exposed to sounds of increasing intensity. The most common reactions were attempts by the subjects to avoid the site of previous noise exposures, or attempts to avoid an exposure in-progress. Schlundt et al.* gave a brief summary of the more significant behavioral changes they observed in dolphins and white whales exposed to intense pure tones. This report presents a more detailed summary of behavioral responses of dolphins and white whales exposed to 1-s tones.

*(Schlundt, C. E., Finneran, J. J., Carder, D. A., and Ridgway, S. H. (2000). "Temporary shift in masked hearing thresholds (MTTS) of bottlenose dolphins, *Tursiops truncatus*, and white whales, *Delphinapterus leucas*, after exposure to intense tones," J. Acoust. Soc. Am. 107(6), 3496–3508.)

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